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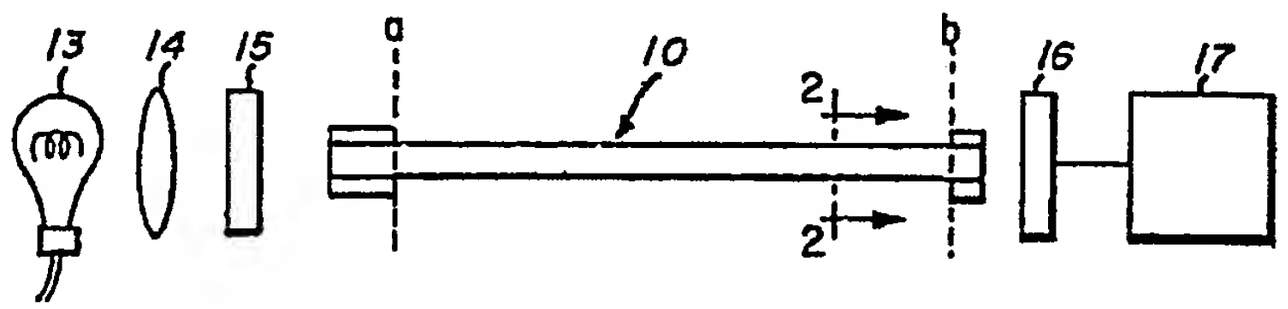

(57) Abstract

Method and apparatus (10) for chemical and biochemical analyses which employ an energy transmissive core (11) and may employ one or more sheaths (12) which selectively absorb, react with, and/or filter an analyte or a product of an analyte. The core is transmissive to a chosen energy carrier and it has an inlet end and an outlet end. Between these ends it has an extended length. The passage of energy through the core is modified by reason of events which occur in one or more of the sheaths or in the case where no sheath is employed by reason of events which occur in an ambient fluid. The resulting modification of the transmitted energy is a measure of such events which in turn are a measure of the analyte. The energy may be any of several types of energy which can be transmitted through the core from end to end and which is susceptible to modification by reactions in the sheath or sheaths or ambient fluid. The energy may be electromagnetic, electrical or sonic. In the method aspect of the invention, a permeable core may be used which is bare, i.e. without a sheath, and exposed directly to an environment, e.g. the air or an industrial fluid.

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METHOD AND APPARATUS FOR ANALYSIS

Many analytical techniques have been developed for chemical or biochemical purposes. Procedures that use a discrete fluid sample for analysis of a single analyte are traditionally characterized as wet chemical techniques or dry chemical techniques. In recent years both types of techniques have been automated in order to reduce costs and simplify procedures. Wet chemical methods, typified by the technicon auto analyzers, utilize batches of reagent solutions, pumps and fluid controls, coupled with conventional sensors, (such as densitometric, fluorescent, colormetric (i.e. radiometric), polarographic, conductimetric, or ultrasonic). These techniques are characterized by large equipment, generally expensive, and generally requiring a skilled operator.

Dry chemical techniques utilize reagents stored under dry conditions within a single or multi-layer flat element such that a test liquid will result in a reaction that can be radiometrically detected (see U.S. Patent No. 3,092,465). These techniques are simple to use, but have traditionally yielded only qualitative results. There are several reasons for this that have been well explained in recent U.S. patents assigned to Eastman Kodak (see U.S. Patent Nos. 3,992,158; 4,042,335 and 4,066,403). The major reasons are: non-uniform spreading of the fluid over the flat surface; non-uniform penetration of the fluid or analyte into the region where the reagent is stored; and non-uniform effects at the edges of the spread liquid. The well-known "dip stick" products had to utilize chemical reactions that proceeded to completion because their transient response characteristics were temperature dependent and they were used in a non-thermostatted environment. A new system of dry chemistry has been

recently intrcduced by Eastman Kodak claiming to overcome many of the traditional weaknesses of dry chemical methods.

The Kodak technique utilizes flat, multilayered sheets arranged in sequence such that the top layer receives the liquid sample and it passes downward undergoing separations and reactions in a pre-arranged sequence. The sheet is designed to accept a small volume of liquid and distribute it uniformly over a reproducible area; the area is less than the total area of the multilaminar sheet. Each layer of the sheet is essentially homogeneous in a direction parallel to the surface; thus, once spread radially (a rapid process) the components of the liquid can move downward at rates that are essentially the same in any plane that is parallel to the surface. In this way uniform reactions, filtrations, etc. can occur.

The analyte is detected in such multilayered sheets by radiometric methods, carried out in a thermostatted environment. This permits one to use kinetic measurements as well as static ones in order to detect analyte concentrations in the liquid sample.

Radiation is caused to enter this assembly in a path which is transverse to the several layers. The radiation is modified by the analyte or by a component or product of the analyte. For example, the exciting radiation may be partially absorbed by the analyte or by a component or product of the analyte. The modified radiation may be reflected back transversely through the laminar assembly or it may pass through the entire assembly. In either case (reflection or transmission) the path of the exciting radiation is very short and is determined by the thickness of the layer in which the

exciting radiation encounters the substance which is excited. Since this dimension must be very small to permit rapid measurement, e.g., 10 μm to 100 μm the degree of modification of the exciting radiation is quite small. This limits the applicability of this technique to analyses wherein the analyte (or a component or product of the analyte) interacts very strongly with the exciting radiation or it requires the use of very sensitive detecting apparatus. It has been shown to be a useful method for measuring analytes in blood that exist at relatively high concentrations, e.g., glucose, BUN, cholesterol, albumin.

Other analytical methods have been developed that utilize rapidly reversible chemical reactions in order to continuously monitor analyte concentrations in biological fluids, or industrial effluent streams, or ponds, lakes and streams. For example, several methods have been proposed to measure the oxygen level in blood of critically ill patients.

It is an object of the present invention to provide improvements in analytical procedures and apparatus.

It is a further and more particular object of the present invention to provide an analytical technique (both method and apparatus) by which a laminar assembly of the general type described above can be employed without the limitations inherent heretofore.

The above and other objects of the invention will be apparent from the description below and the appended claims.

In accordance with the present invention a core is provided which is transmissive to the chosen

energy, which may be electromagnetic (e.g. ultraviolet or visible light), electronic or sonic energy. This core is provided with one or more permeable or semi-permeable sheaths (i.e. permeable to a fluid sample containing the analyte, or permeable to so much of the test fluid as is desired but acting to filter out unwanted components). This part of the apparatus may be referred to as the "sheath structure" signifying that it may consist of one or more permeable or semi-permeable sheaths. As will appear more fully from the description below, there are several functions that may be performed by the sheath structure any one or more of which may be performed by one or more of the individual sheaths.

The core, as stated, is transmissive to the chosen energy and causes that energy to pass in a direction generally parallel to the surface to which the test fluid is applied. The core has an "active length" which, as will appear more fully hereinafter, is that portion of its length usually, but not necessarily, less than the entire length of the core, wherein the energy passing through the core is subjected to the influence of the test fluid and is modified thereby. The magnitude of this active length is large, and as will appear more fully hereinafter it is very large compared to the thickness of the sheath structure and/or the core. By this arrangement the energy passing through the core along its active length is subjected to a cumulative, although not necessarily uniformly cumulative, modification by reason of the presence of analyte in the test fluid.

As will also appear more fully hereinafter, the core, if permeable to an analyte in a liquid or gaseous test fluid, or to a product of such analyte, may be bare, i.e. devoid of a sheath structure and may therefore be in direct contact with the test fluid.

The physical shape and configuration of the device or apparatus of the invention may vary considerably. In a preferred embodiment, the device is cylindrical and consists of a central core or fiber which is transparent to the exciting radiation and is surrounded by a sheath structure consisting of one or more concentric layers of absorptive material. Alternatively the shape in cross section may be polygonal with one or more absorptive layers surrounding the transmissive layer.

In any such configuration, the path of the carrier energy is generally parallel to, rather than transverse to the overlying layer or layers. Many of the figures in the drawings will serve to illustrate various embodiments of the invention. For the most part the carrier energy will be described as electromagnetic radiation.

In Figure 1 there is shown one form of the apparatus of the present invention which is generally designated by the reference numeral 10 and which, as shown in the cross-sectional view of Figure 2, consists of an inner core of fiber 11 and an outer sheath 12. The core or fiber 11 is transparent to the exciting radiation but, instead of being a conventional optical fiber such as quartz fiber, it is selected so that it is not only optically transmissive but is also permeable to components of an aqueous solution. The outer sheath 12 is of absorptive, semi-permeable material.

To the outer sheath 12 is applied, for example by immersion or dipping, a fluid containing the analyte under consideration, for example blood where it is desired to measure one of its low molecular weight components. If it is desired to prevent penetration by

large molecules and formed elements such as erythrocytes, platelets, or white cells, the material of which the sheath 12 is constructed may be suitably selected so as to filter out such large molecules and elements. Alternatively an outer layer (not shown) may be provided which is permeable to water, and small molecules but which is impermeable to large molecules and formed elements of the blood. Thus, it is understood that one function of layer 12 is to act as an impermeable barrier to those unwanted components of the test fluid.

When the device 10 is immersed in the test fluid, for example, in blood, the fluid will penetrate to the core of fiber 11. Its presence may be detected by illuminating the core with (e.g.) light of a wavelength that is selectively absorbed by the analyte. Thus the diminution of light that emerges from the exit end of the fiber is proportional to the concentration of the analyte in the sample fluid.

In Figure 1 there is shown diagrammatically a system for carrying out such a determination of analyte in an aqueous solvent, including a source of radiation 13, a focusing lens 14 and a suitable filter 15 to transmit light of the proper wavelength. At the exit end of the device is a light detector 16 and an electrical signal processor 17. The electrical signal processor amplifies the signal from the light detector and may be made from any of several well known and commercially available devices, and may include readout means of visual type and a recorder for a printed readout.

It is to be understood that the instruments of Figure 1 that are used to introduce energy into the analytical apparatus and to measure that which leaves the system can be configured in any of a number of ways

depending on the use of the device. The simplicity of use and intrinsically rapid response of the invention suggests that one use will be to make bedside measurements. In such a case a portable unit with rechargeable batteries would be preferred. It is to be further understood that modern electronic data processing methods are so compact that one may further simplify the use of the analytical apparatus by utilizing electronic corrections and calibrations which permit the use of assay methods that are non-linear in their response to the analyte concentration. In fact, it is possible to assemble several analytical elements in a parallel arrangement in a single instrument.

The letters "a" and "b" and the lead lines therefrom in Figure 1 signify the "active length" of energy transmissive device 10. This active length is that portion of the device which is exposed to analyte, or to a product of an analyte, and along which the flow of energy is cumulatively modified by the analyte or a product of the analyte. The path from "a" to "b" (which may be continuous or segmented) is long compared to the thickness of the element 10.

In the apparatus of Figure 1 the core 11 may be bare, i.e. devoid of a sheath. Thus where, for example, the atmosphere or water, an industrial fluid or a biological fluid contains the analyte of interest, e.g. a contaminant such as sulfur dioxide or a nitrogen oxide in stack gas or a phenolic contaminant, the material of the core 11 may be selected so that it absorbs such contaminant which modifies the flow of energy through the core, e.g. by absorption of a selected wavelength of light.

Referring now to Figure 3, a system similar to that of Figure 1 is shown where the fiber assay device

(FAD) 10 shown on a larger and exaggerated scale and with the interior exposed to show the several components including a core 25 (hereinafter designated by the letter "C") which is transmissive to ultraviolet radiation and is impermeable to fluids; and a sheath 26 of gas permeable material containing an oxygen quenchable fluorescent dye substance. The index of refraction n_s of sheath 26 is greater than the index of refraction n_c of the core. The outer sheath 27 is oxygen permeable and is reflective. It may be impermeable to large molecules and to formed elements such as platelets and red cells in blood.

Referring to Figure 4, which is a fragmentary cross section through the FAD, molecules of fluorescent dye are indicated at 28. Ultraviolet light passes through the core and some of it is refracted into the sheath 26 where it reacts with fluorescent molecules which emit radiation in the visible spectrum; such emission is isotropic. That visible radiation which impinges upon the interface between the sheaths 26 and 27 is reflected into the core along with radiation which enters the core directly. Assuming, as will be the case in practice, uniformity of distribution of fluorescent molecules in the sheath 26, during passage through each increment of length of the core ΔX, (X being the distance along the active length of the core) the radiation passing through the core will pick up an increment Δi of emitted light. Δi will not, of course, be constant inasmuch as the UV radiation is somewhat attenuated as it passes through the core 25. Nevertheless a cumulative effect will occur and the intensity of emitted visible light $\sum_{0}^{x} \Delta i$ will be much greater than the value of Δi emerging from a small segment of the path.

When a fluid, for example blood, industrial water or river water containing oxygen (which is the analyte to be determined) is applied to the outer sheath 27, it will penetrate to the sheath 26. The dissolved oxygen in the fluid will quench fluorescence, therefore, it will diminish the intensity of fluorescent light emitted at the output end of the core. The emitted radiation passes through the ultraviolet filter 18, then through an opto-electrical detector (O-ED) 30 which acts as a transducer to convert the emitted light energy into electrical energy. The emitted electrical energy is processed in a unit 31 resulting in a digital or analog output. A suitable processor consists of amplifiers, limiters, meters, and elements for electrical logic, as are well known to those in the instrumentation business. A molecule well known for its tendency to exhibit O_2 quenching of fluorescence is fluoranthrene.

Figure 5 shows a curve typical of such an output. Advantageous features of this system include its low temperature sensitivity, and rapid response time.

Referring now to Figure 6, a plot of analyte concentration against output of a similar system is shown.

The solid curve represents a mean calibration curve in the absence of other molecules. The dotted curve is the calibration curve in the presence of a molecule that may also occur in the fluid. Thus, the calibration curve shifts up or down the vertical axis as a function of the unknown concentration of contaminant, C_C. If C_A is the concentration of the analyte, we may represent this phenomenon of interference by the expression:

$$\text{OUTPUT} \propto (C_A + C_C)$$

where α represents a proportionality. In order to obtain an output that is independent of C_c one would traditionally have to remove C by some chemical process, or measure it by an independent method. However, the present invention lends itself to a double fiber assay (DFA) system which dispenses with the need for calibration, such being shown in Figure 7. As shown in Figure 7 there are two FAD units one of which 33 measures the output shown in Figure 5; the other being a unit 34 which is designed so as to measure the concentration of contaminant C. The output of the two fibers can be detected with equal sensitivity because the fibers are illuminated from a single light source. The two signals can be electronically subtracted as can be done in commercially available analog or digital devices indicated generally at 35. Therefore without the need for calibration the output is a measure of the concentration of analyte.

Yet another limitation of many highly sensitive assay systems (e.g. radioimmune assay) is summarized in Figure 8. The analyte is detected by reaction with a reagent. The high specificity of the reaction leads to a steep dose response curve; i.e. for a given reagent concentration the output changes over its full range when traversing a narrow range of analyte concentration. When the range of anticipated analyte concentration is wide, as denoted by the shaded area in Figure 7, one must perform several determinations, each with a different reactant concentration in order to determine the analyte concentration.

Referring now to Figure 9, a multiple fiber assay device (MFAD) is shown consisting on n such FAD devices, each of which embodies within one of its sheaths a reagent. The reagent concentrations vary from

one device to the other. It will be apparent that when these fibers are wet by a fluid that there is one reagent concentration resulting in an output which is a reflection of the analyte concentration. The other fibers either over- or under-react with the analyte yielding unmeasurable outputs. Assume that FAD_j (j being an integer from one to n) is optimum. The separate outputs of the FADs are converted by O-EDs to electrical outputs which are separately transmitted to a switching device 36 controlled by a microprocessor 37 to select the output of FAD_j and reject the others.

Referring now to Figure 10, a dual FAD system is shown intended for the determination of the glucose concentration of a body fluid, for example blood. As in Figure 3, sheath 26 of each device contains an oxygen quenchable dye and sheath 27 is an oxygen permeable sheath which reflects light back into the core. The sheath 27 also functions to prevent penetration of fluorescent molecules from the sample. Alternatively, an outermost sheath (not shown) may be employed for that purpose. One such device 36a is the control device that measures oxygen (the contaminant); and the other 36b is modified by having in the sheath 27 a quantity of glucose oxidase. Both devices are wetted with a sample simultaneously. Oxygen in both samples penetrates through sheath 27 to sheath 26 and quenches fluorescence. However, glucose oxidase in sheath 26 of device 36b causes reaction of a portion of the oxygen with the glucose and therefore diminishes the oxygen available for quenching fluorescence. The rate of oxidation is proportional to the concentration of glucose. Therefore, the output of the device 36b will be greater than that of the device 36a and the difference is measured by the output of the device. The system includes a filter 37, OED devices 38 and a processor 39.

Referring now to Figure 11, the output of each O-ED in Figure 10 is plotted as a function of time following addition of a blood sample to the DFAD, presuming that the oxygen level of the blood is higher than that of ambient air. In the case of the oxygen sensor 36a the excess oxygen is lost by diffusion to the air and ultimately the output (dotted) curve reaches a steady value of fluorescence that reflects equilibration with air. However, the modified fiber 36b consumes the oxygen more rapidly due to its reaction with the glucose in the sample, causing a greater rise in fluorescence. When all the glucose that is present in the blood has been consumed, this fiber also equilibrates with ambient air. Thus, the two curves ultimately merge. Figure 12 is a curve representing the integration of the space between the two curves and therefore its area is a measure of the total amount of glucose in the sample.

Referring now to Figure 13, a plot is shown in the case of continuous monitoring where the dual FAD device is implanted within a patient. (See also Figures 22 and 23 below and the description thereof). The first portion of the curve represents normal variations in glucose level in the blood and the large increase represents a large increase in glucose level after, for example, a patient has had a meal. The solid curve represents the output of device 36b and the broken line curve represents the output of device 36a. Figure 14 is a plot of the difference between the curves of Figure 13, therefore of the variation with time of the glucose level of the patient.

Referring now to Figure 15, a single FAD of a multiple FAD system is shown as an example of an immunoassay described in Figure 9 above. The FAD comprises an innermost sheath 41 whose index of refraction n_s is

greater than the index of refraction n_c of the core and which contains an antigen-fluorescein complex designated as A* in quantity sufficient to fully saturate an antibody located in sheath 43. Sheath 42 is a sheath which is hydrophilic and contains reflective particles and which is impermeable to antigen (A) when dry but permeable when wet. Sheath 43 is hydrophilic and contains as a reagent, an antibody Ab to the antigens A* and A. (A is the analyte of interest.) The outermost sheath 44 is microporous. A sample of fluid containing the antigen A is added to sheath 44.

The kinetics of diffusion and reaction of the system are as follows:

A diffuses through sheath 44 into sheath 43 and A* diffuses through sheath 42 into sheath 43. The following competing reversible reactions occur in sheath 43.

(1) $A^* + Ab \rightleftharpoons A^* - Ab$

(2) $A + Ab \rightleftharpoons A - Ab$

The antibody complexes A*-Ab and A-Ab cannot diffuse out of sheath 43. There is enough A* in sheath 41 to saturate the Ab in sheath 43. Assuming the case of no antigen A in the sample, reaction (1) will proceed to a state of equilibrium at which time the rate of diffusion of A* out of sheath 41 will equal the rate of diffusion of A* back into sheath 41. At that time the output will become constant, as shown by the lower curve in Figure 16. Virtually all of A* is bound to Ab, yielding a low level of fluorescence. The middle curve represents the case when the sample contains a finite quantity of A; and the upper curve represents the case where A>>A* so that very little Ab-A* is formed.

The spacing of the curves in Figure 16 is arbitrary. It will be apparent that in actuality this spacing will depend upon the concentration of A* in sheath 41 and the concentration of A in the sample. By employing a bundle of FADs each containing a different concentration of A* in sheath 41, one of the devices will contain an optimum concentration of A* such that the spacing of the curves is optimum. By means of the logic selector 45, the output of that device will be selected and the others rejected. By calibration the concentration of A in the sample may be determined.

Yet another method for utilizing the specificity and high sensitivity of immunoassays is shown by Figures 17 and 18. Figure 17 illustrates in detail a single element of an array of several FADs, each containing different concentrations of antibody Ab. The core c is permeable to the product Y of a reaction

$$X \xrightarrow{E} Y$$

wherein E is an enzyme, X is high molecular weight and Y is low molecular weight. Sheath 50 is permeable to Y but not to X; sheath 51 contains an antibody Ab and an antigen-enzyme complex A-E wherein the enzyme E is active. This complex reacts with antibody in accordance with the following reversible reaction.

$$A - E + Ab \rightleftharpoons A - E - Ab$$

Enzyme E is inactive in A - E - Ab. Sheath 50 has a lower index of refraction than the index of refraction of core c, in order to reflect all the light along the axial path within the core.

This system is intended to measure analyte A, which is an antigen, in a fluid sample. Sheath 51 is microporous and permeable to A. When sheath 51 is wetted with a sample containing the antigen A, it diffuses into sheath 51 but no further. There it reacts with Ab, displacing A-E in direct proportion to their relative concentrations. The A-E that is released catalyzes the reaction, forming Y which then diffuses into the core where it is detected by light that is transmitted along the core. If the wavelength of light is selected for maximum absorbance by Y and sheath 51 contains an excess of X the method will be highly sensitive and specific for the presence of A in the sample fluid. Figure 18 describes the electrical output of the system following the application of the sample to sheath 51. As in Figure 9 this analytical method will require the use of a switching system so as to measure the curve for the element that contains the optimum quantity of Ab-A-E. The rate of change of output from that element will then be proportional to the concentration of A in the sample.

Referring now to Figure 19, an FAD is shown comprising a core c and a sheath 52 having a lower refractive index than the core or containing reflective material (or surrounded by a reflective sheath, not shown). The sheath 52 is permeable to analyte (i.e. an antigen, A) but not to higher molecular weight components of the sample. The core contains antibody Ab bonded to a dye D to form the complex Ab-D. Light at the absorption peak of the dye is transmitted through core c. The dye exhibits the property that its absorptive powers are changed when the antigen binds to Ab-D. When sample is applied to sheath 55, antigen A (the analyte of interest) diffuses through this sheath and into core c, which is selected for this purpose. The reversible reaction

$$Ab\text{-}D + A \rightleftharpoons Ab\text{-}D\text{-}A$$

occurs.

This causes a shift in the amount of light transmitted through the element. By using a set of elements with varying concentrations of Ab-D one may select the optimum element for the level of A in the fluid. Since the reaction of A with Ab-D is reversible this may be used for continuous monitoring.

When measuring the analyte concentration in a discrete fluid sample, it is necessary to apply a reproducible volume of fluid uniformly to the analytical element described herein. While this can be achieved by a skilled operator utilizing micro pipettes, it is intended here to describe another means whereby this may be achieved in a simpler fashion. It will be seen that this is a unique feature of the elements described herein.

Referring now to Figure 20, a means for uniformly saturating an FAD is shown which employs the phenomonen of wicking. The FAD shown consists of a core c and a single sheath S. The same means may be employed with FADs containing several sheaths and two or more FADs.

A base 60 is shown comprising a rigid support 61. Adhered to the top of the base is a hydrophilic coating 62 which is wetted with the sample which is used in excess to that needed to wet the FAD. The sample fluid will diffuse through the base to the FAD. It will ascend by capilliary action to the top of the FAD. Provided the height of the device is not excessive, the wicking or capilliary effect will result in a uniform wetting of the sheath S.

The analytical device described herein may be adapted to permit the measurement of large molecular weight analytes that do not normally permeate sheaths in a selective fashion. Figure 21 describes such a device that is useful for determining the quantity of enzyme in a fluid sample. The core c is permeable to a dye D and is transmissive to light with a wavelength selected for maximum absorption by D. The dye D is chemically bound to a hydrophilic polymer that is incorporated into sheath 57. Sheath 58 is permeable to D, impermeable to higher molecular weight components of the fluid, and selected from materials with a lower refractive index than the core. The chemical bond between the dye and the polymer will be selected so it can be selectively degraded by the enzyme in the fluid that one wishes to detect. Thus, for example, if one wishes to measure the concentration of esterase in plasma, utilize an ester linkage between the dye and the polymer. This can be accomplished e.g. by use of any of the dyes classed as acidic dyes, reacted with the hydroxyl groups in gelatin.

When the element is wet by a sample, D will be enzymatically released and diffuse into the core where it will be detected by a decrease in light transmission through the element. The rate of change in light transmission will be proportional to the concentration of the enzyme.

One of the advantages of the fiber embodiment of the invention is that it can be readily incorporated in a catheter for insertion into the body, for example, into a vein or an artery. A suitable form of catheter-fiber structure is shown in Figure 22.

Referring to Figure 22, a catheter 70 is formed in two parts, namely a tip portion 71 and a body

portion 72. These are made of suitable material such as polypropylene, polyethylene, silicone rubber, polyvinyl chloride, or poly (ethylene vinylacetate) and may have a diameter of, for example, .5 to 1.5 mm, appropriate for a particular purpose such as, for example, insertion into a vein or an artery. A cylindrical fiber-shaped device 73 is affixed, for example, in a spiral configuration as shown, to the tip 71 such that it is exposed to the body fluid when the catheter is implanted. The fiber 73 has protruding tips 73a and 73b. The exposed fiber 73 (either all or a portion of it) is susceptible to penetration by an analyte and is of a construction such as that shown in any of Figures 2, 3, 7, 9, 10 or 19, hereinabove. The body 72 of the catheter is formed with parallel passages 75 into which fiber extensions 73a and 73b are inserted, these being recessed so as to form sockets 77. The fiber extensions 73a and 73b may be bare optical fiber or they may be coated with a protective coating. When the tip 71 and the body portion 72 are assembled in operative condition the protruding tips 73a and 73b will be received in the sockets 77 and will be in physical and optical contact with the fiber extensions 75a, thereby providing a continuous optical path from a source of exciting radiation to the output end.

With such a catheter continuous monitoring of a body fluid is possible with an appropriate readout to inform the diagnostician, either visually or by printout or by both means.

Referring now to Figure 23, another form of catheter is shown which comprises a housing 80 through which an optical fiber 81 passes and which also supports an extension 82 of the optical fiber having a portion 82a exposed to body fluids. The exposed portion 82a may

be constructed as in any of Figures 2, 3, 7, 9, 10 or 19 above. The projections 81a of fibers 81 and 82, project into sockets 83 in a tip 84 which embodies a prism 85. When the tip 84 and the housing 80 are brought together a continuous light path is provided through the optical fiber 81, the prism and the optical fiber 81a.

General Discussion

It will be apparent that the invention may be manifested in numerous forms and is capable of numerous applications. Structurally there are at least two elements one of which, exemplified by the transmissive core is a medium for transmitting energy in continuous form such as electro-magnetic energy, e.g. ultraviolet light or visible light, electric current (AD or DC) or sonic energy. The other element (or elements) is a sheath or sheaths. The configuration is preferably rod-like with a transmissive core in the form of a fiber typically about 10 μm to 1 μmm in diameter with one or more sheaths surrounding the core typically about 10 μM to 100 μM in thickness. The active length of the device (i.e., the length which is wetted by the test fluid) may vary from about 0.5 cm or less to 1 meter or more. In most bioassay applications the length will not exceed about 10 cm. Departures from such dimensions are permissible. As stated above, other configurations, e.g. polygonal configurations, are permissible.

The core, besides its transmissivity and shape, may have the following characteristics: It may be impermeable to aqueous liquids. If permeable it may contain a reagent, e.g. a dye, or it may be devoid of a dye and be a receptor for permeation by a reagent. Suitable materials for impermeable cores are quartz, polymethylmethacrylate, polycarbonate, polystyrene, etc. If the core is permeable, suitable materials are plasti-

cized polyvinyl chloride, polyurethanes, polypropylene, nylon, gelatin, polyvinyl alcohol, natural rubber, butyl rubber, cis-polyisoprene, poly (ethylene vinyl acetate). The index of refraction of the core, n_c, may be greater or less than that of the adjacent sheath.

The material and construction of the sheaths will depend upon their function. In all cases when a reaction occurs in a sheath or where a liquid must diffuse into, through or out of a sheath, it should be permeable to water. Permeable sheaths may be permeable to large and small molecules and to finely divided solids suspended in a liquid sample, or they may be selective with regard to permeability such that unwanted large molecules, etc. are excluded, as taught in the book by Crank and Park, "Diffusion in Polymers." One or more sheaths may contain a reagent or a precursor of a reagent and such reagent or precursor may be immobile or mobile, and it may undergo a reaction such as enzymatic cleavage to render its product mobile, or it may undergo a reaction such as antibody-antigen reaction which makes it immobile. All such physical states of sheath material and reagents are possible and methods of synthesis or forming are well known to those practiced in the art.

Elements of the dimensions suitable for this invention e.g. (1-100 μm diameter) can be made by normal fiber-forming techniques; the Encyclopedia of Polymer Science and Technology provides an adequate description of these techniques. Briefly, there are three major techniques: melt forming, wet forming, and dry forming. Melt forming is used for thermoplastic polymers (e.g. polypropylene) that exhibit a low viscosity when heated above their melting point. Wet process forming consists of extruding a solution of the polymer in a solvent and

passing the fiber through a bath of a second solvent. This bath solvent has the property that it will dissolve the polymer solvent, but not the polymer; thus the solvent is extracted by the bath solvent, leaving a pure polymer fiber. Dry forming consists of extruding a solution of polymer and volatile solvent into a heated air stream, where the solvent evaporates.

Adaptations of these processes can be used to coat the core fiber with the sheaths. If the central fiber is made from a high melting point material (e.g. glass), one could coat it with a melted polymer. It is more likely that one will use polymer solutions, especially when it is necessary to incorporate chemicals that are used to react with analyte or otherwise participate in the required chemical analysis. Many such reagents degrade under conditions of high temperature.

If, for example, one wanted to form an assymmetric microporous membrane as the outer sheath as is required for the enzyme assay described above (Figure 21), one could use wet forming. The polymer and dye-polymer conjugate would be dissolved in a solvent and coated onto the fiber by pulling the fiber (c, coated with sheath 58) through an orifice that has the solution on the upstream side. On the downstream side is a bath with a solvent that is selected to elute the primary solvent leaving a polymer sheath 57 that has microscopic holes. The bath solvent is selected so as to be non-solvent for both the polymer and the dye-polymer conjugate.

Once formed as a continuous, long coated fiber, it will be cut into short lengths and mounted in a holder, whose purpose is to align the ends of the fiber into a reproducible position, to provide a simple

means to insert the fiber into the instrument (cf. Figure 1), and a means to protect the fiber during storage and use. The housing could also be within a catheter for use as a monitoring instrument as shown in Figures 22 and 23. The catheter could be inserted into the fluid or body cavity of interest; it contains highly conductive input and output fibers that are coupled to each end of the coated fiber assay system so as to introduce exciting radiation and to recover the analyte-modified radiation. These housings will generally be made of plastic material and are understood to be fabricated by the standard methods available, namely, injection molding, transfer molding, extrusion, epoxy molding or heat forming.

Reagents, reagent pre-cursors, reflective material, etc. which may be incorporated in various sheaths include the following: enzymes, O_2-quenchable fluorescent molecules (e.g. fluoranthrene), antibodies, dyes, fluorescent dyes, reflective materials (TiO_2, SiO_2, etc.) dye-polymers products. These and other reagents, reagent pre-cursors and reflective materials are well known to those skilled in the art.

It will therefore be apparent that new and useful apparatus and methods are provided for chemical and biochemical analyses.

CLAIMS

1. Apparatus for analysis comprising:

(a) an energy transmissive element having a longitudinal dimension and a transverse dimension and capable of transmitting energy along said longitudinal dimension,

(b) a source of energy capable of transmission through said element along its longitudinal dimension, such energy being selected so as to be capable of modification by the presence of an analyte contained in a fluid external to the element,

(c) an energy sensing and processing means capable of detecting and measuring the change of transmitted energy resulting from such modification,

(d) said element (a), source (b) and means (c) being arranged so that energy from the source is transmitted through the element (a) along its longitudinal dimension in a path wherein the transmitted energy is modified by the presence of analyte in an ambient fluid and so that the modified energy emerging from such path is sensed and measured by said means (c),

(e) said path being sufficiently longer than said transverse dimension that there is a rapid response of modification of transmitted energy to the influence of ambient analyte, such modification being cumulative along said path.

2. The apparatus of claim 1 wherein said element is permeable to the analyte, to the product of an analyte or to a reagent.

3. The apparatus of claim 2 wherein said element is directly exposed to ambient analyte which permeates the element and thereby causes modification of transmitted energy.

4. The apparatus of claim 1 wherein said element (a) is surrounded by a sheath structure includ-

ing at least one permeable sheath, and the apparatus includes a reagent which is reactive with an analyte or with a product of an analyte.

5. The apparatus of claim 4 wherein the reagent is incorporated in the sheath structure.

6. The apparatus of claim 4 wherein the reagent is incorporated in the element (a).

7. Analytical apparatus comprising:

(a) a first lamina

(b) a second lamina surrounding and in operative contact with the first lamina

said first lamina being transmissive to energy capable of modification by events during analysis occurring in or transmitted through said second lamina,

said second lamina being permeable to aqueous liquids and at least certain of their dissolved components

the configuration of said apparatus being such that the dimension perpendicular to the interface of the laminae is small compared to the dimension parallel to such interface

said second lamina being selected from a material which provides a zone for occurrence of events, or for transmission of events, resulting from wetting of the apparatus with a fluid sample containing an analyte, such events being capable of modifying the energy transmitted through said first lamina

such modification being cumulative as energy is transmitted through the first lamina.

8. The apparatus of claim 7 wherein the first lamina is impermeable to aqueous liquid.

9. The apparatus of claim 7 wherein the first lamina is permeable to small solute molecules.

10. The apparatus of claim 8 wherein the second lamina contains a reagent which is reactive with the analyte or with a product of the analyte.

11. The apparatus of claim 7 wherein the first lamina is transmissive to electromagnetic energy and the apparatus includes in one of its laminae a reagent which is reactive with an analyte or with a product of an analyte to result in a product which modifies the output of electromagnetic energy by the first lamina.

12. Analytical apparatus comprising:

(a) a core which is transmissive to energy

(b) a sheath structure including at least one permeable sheath surrounding and in operative relation to said core,

said core providing a path for transmission of energy which is large compared to the thickness of elements (a) and (b), and

(c) a reagent embodied in said apparatus of a nature and so located that upon wetting of the apparatus with a fluid sample containing an analyte, a product resulting from direct or indirect action of the analyte upon the reagent causes a modification of the energy passing through said core, such modification being a measure of the concentration of the analyte and being cumulative along said path.

13. The apparatus of claim 12 wherein the core is transmissive to electromagnetic energy and is impermeable to aqueous liquid and the reagent is located in a sheath surrounding the core.

14. The apparatus of claim 12 wherein the core is permeable to analyte and contains the reagent.

15. The apparatus of claim 12 wherein the core is permeable to aqueous liquid and wherein the reagent is contained in a sheath surrounding the core and results, when acted upon directly or indirectly by the analyte, in diffusion of a substance into the core which modifies the transmitted energy.

16. The apparatus of claim 14 wherein the reagent is a dye.

17. The apparatus of claim 15 wherein the reagent is a dye.

18. Apparatus as in claim 12 including a plurality of successive sheaths one of which contains the reagent.

19. Apparatus of claim 12 wherein the index of refraction of the core is less than that of the adjacent sheath, said adjacent sheath contains a reagent which is acted upon by electromagnetic energy diffracted from the core and reflective means is provided to reflect radiation back into the core including radiation of a different wavelength than incident radiation.

20. Apparatus of claim 19 including an outermost sheath which is microporous and acts to exclude large molecules from the inner sheath or sheaths.

21. Apparatus including a pair of devices as defined in claim 7, radiation input means for separate input of energy of the same type into each device, one such device containing reagent and the other being

devoid of reagent, and means connected to the output of each device to subtract one output from the other to eliminate background output and to measure only modification of output due to analyte.

22. Apparatus including a multiplicity of devices as defined in claim 7 each containing a reagent appropriate for the intended analysis but in different concentrations, together with means for selecting the output of that device whose output is optimum for purposes of the analysis and rejection of outputs of the other devices.

23. Apparatus including a device as defined in claim 12 in the form of a catheter insertable into the body of a patient to monitor a bodily function and including terminals for connecting the input of the core to a source of energy and connecting the output of the core to equipment for measuring the fluctuation of such function.

24. Apparatus including a device as defined in claim 12 and means for uniformly wetting the sheath structure with a sample by capillary means.

25. A method of analysis comprising:

(a) providing an energy transmissive element defining a path for transmission of energy, the length of said path being much greater than the transverse dimension of the element,

(b) causing energy to be transmitted through said path, such energy being selected so as to be modified by the presence of an ambient analyte,

(c) modifying the thus transmitted energy by exposure of the element along said path to the influence of ambient analyte, such modification being

cumulative along the length of said paths, and

(d) sensing and measuring the change of energy emerging from said path caused by such exposure and modification.

26. The method of claim 25 wherein said energy is electromagnetic energy, which is modified by one of the phenomena: absorption, fluorescence, scattering.

27. The method of claim 26 wherein said element is in the form of a core transmissive to electromagnetic energy and is surrounded by a sheath structure which is permeable to the analyte.

28. The method of claim 27 wherein the core permeable and the core or the sheath structure contains a reagent which is reactive with the analyte or with a product of the analyte.

29. The method of claim 28 wherein the reagent is incorporated in the core.

30. The method of claim 28 wherein the reagent is incorporated in the sheath structure.

31. The method of claim 27 wherein the core is impermeable to the test fluid and a reagent is incorporated in the sheath structure which is reactive with the analyte or a product of the analyte.

32. The method of claim 25 wherein the energy transmissive element is in the form of a transmissive core and is surrounded by a permeable sheath structure including at least one sheath and which is permeable to a fluid containing the analyte, there being a reagent

incorporated in the core-sheath structure which is reactive with the analyte or with a product of the analyte.

33. A method of analysis comprising:

(a) providing an energy transmissive core surrounded by a permeable sheath, one such component containing a reagent which is reactive with an analyte or with a product of an analyte

(b) the configuration and the transverse and longitudinal dimensions of the core-sheath structure being such that energy can be transmitted through the core in its longitudinal direction in a path which is greater than the transverse dimension of the core-sheath structure, such dimensions being selected so that when a fluid sample containing an analyte is applied to the sheath there is a rapid response of modification of energy transmitted through the core and such that the modification is cumulative along such path

(c) exposing the core-sheath structure to a fluid containing the analyte

(d) causing energy to be transmitted through the core along its longitudinal dimension, such energy being selected to be modified by events occurring as a result of such exposure and

(e) sensing and measuring the resulting modification of energy.

34. The method of claim 33 wherein two such core-sheath structures are employed, one of which serves to measure analyte plus a contaminant and results in an output which does not discriminate between the analyte and the contaminant, the other such structure serving to measure the contaminant only, said method including processing the output of the two core-sheath structures and subtracting the output of one from that of the other so as to measure the analyte concentration.

35. The method of claim 33 wherein a multiplicity n of core-sheath structures are employed each of which includes a reagent appropriate for the analyte which is to be measured, said core-sheath structures containing such reagent in n different concentrations, said method further comprising so processing the outputs of the n core-sheath structures that the output of structure j (j being an interger from 1 to n) is selected and the other outputs are rejected, the output of structure j being most suited to measurement of the analyte.

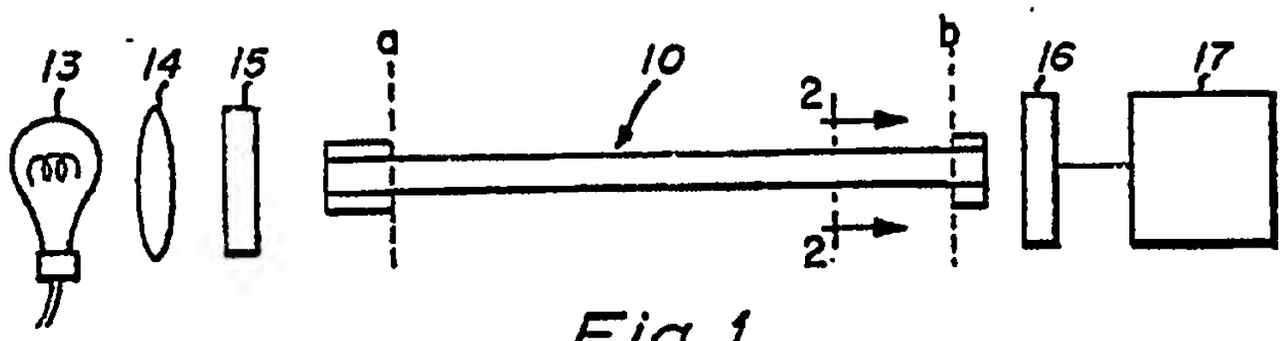

Fig_1

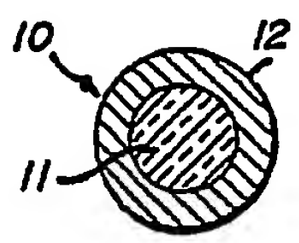

Fig_2

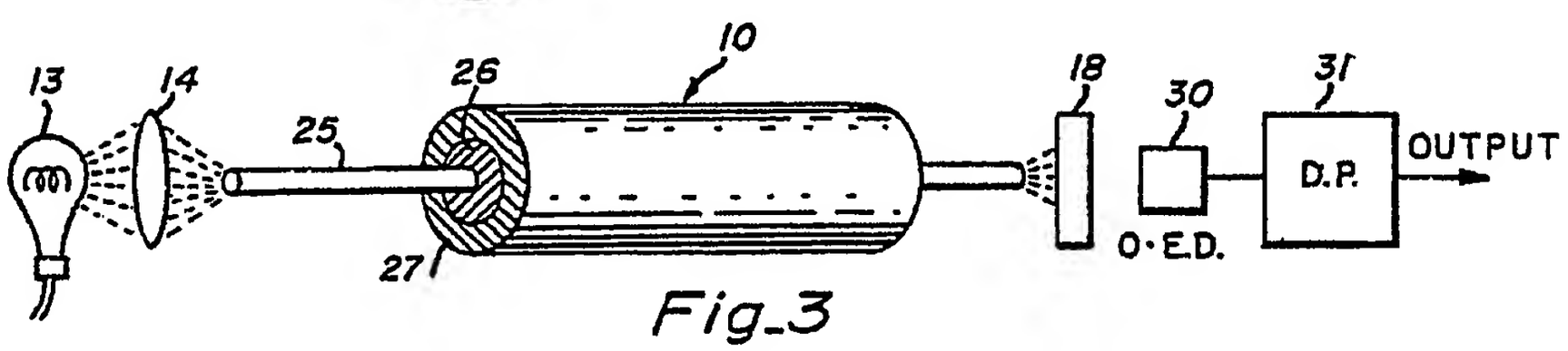

Fig_3

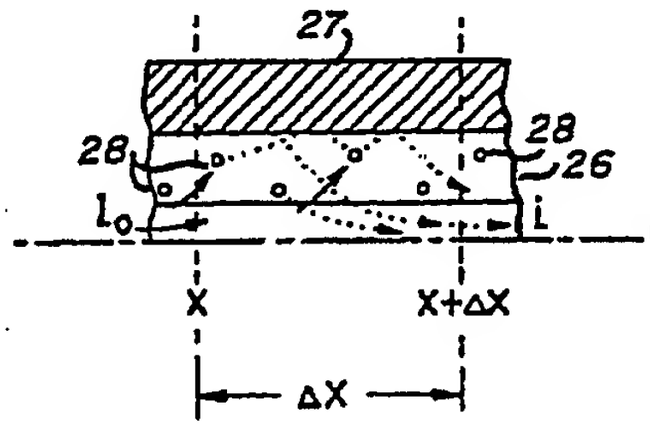

Fig_4

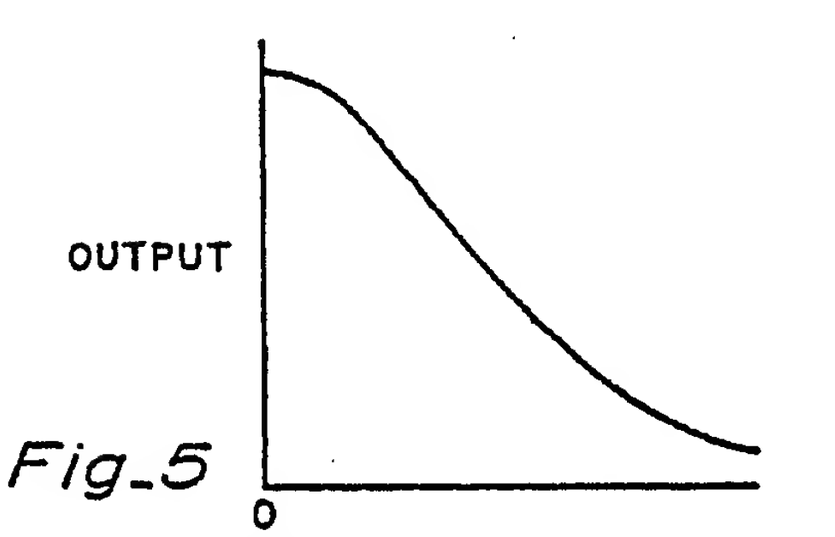

Fig_5

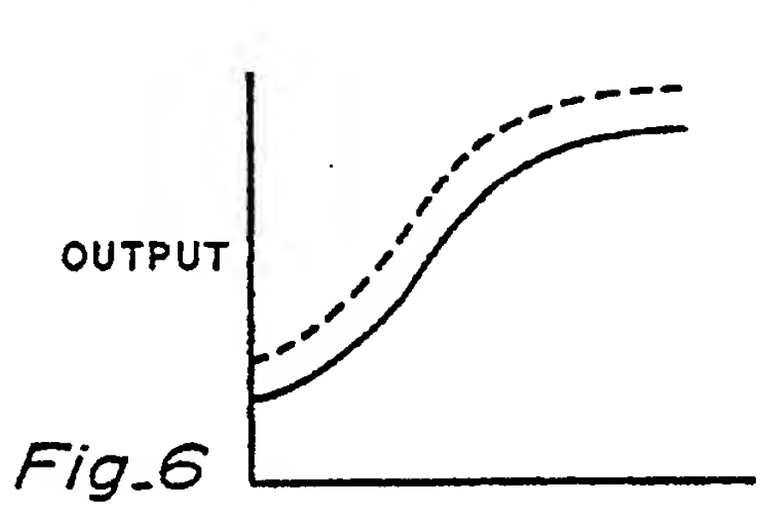

Fig_6

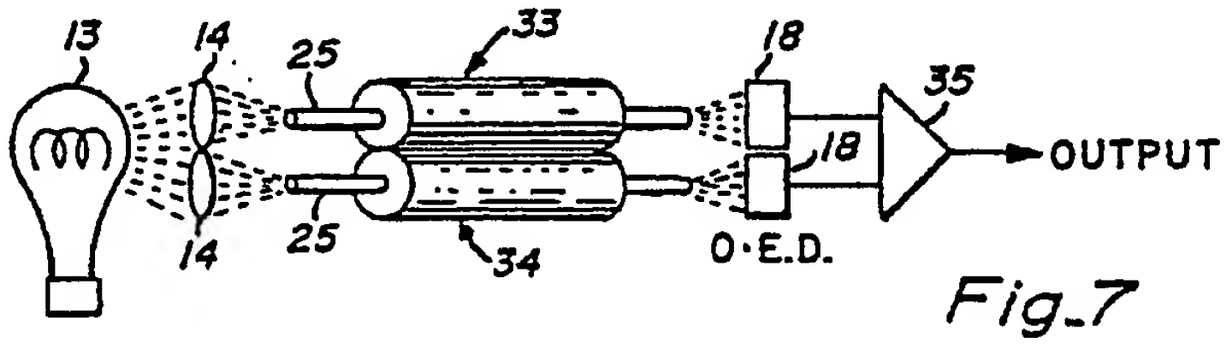

Fig_7

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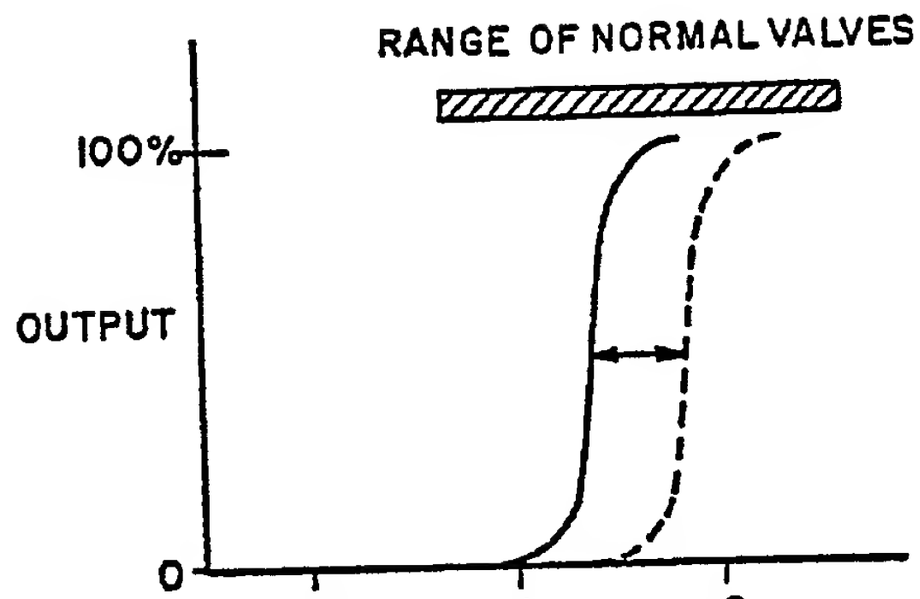

Fig_8

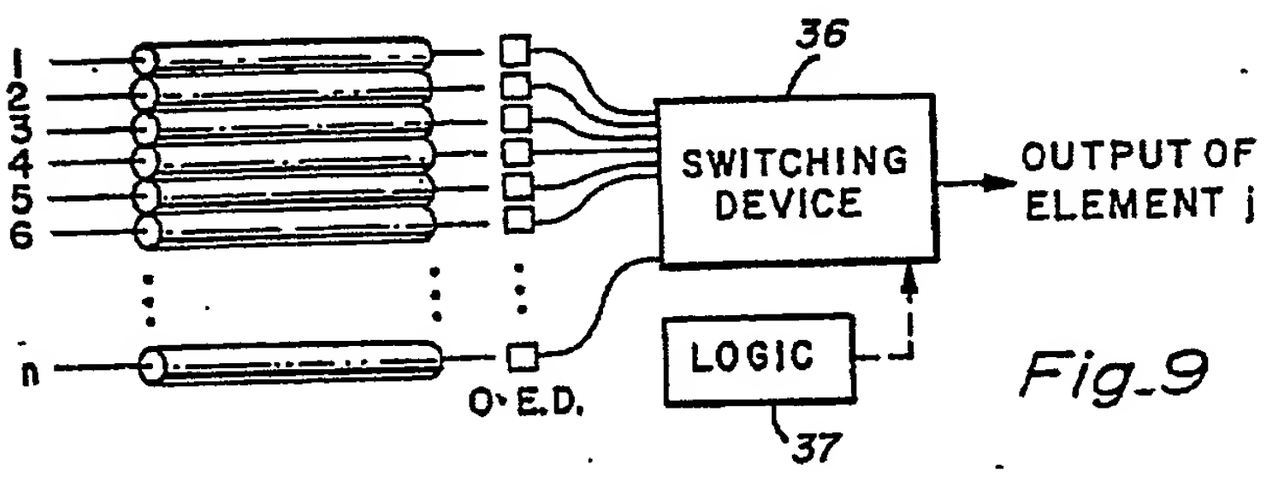

Fig_9

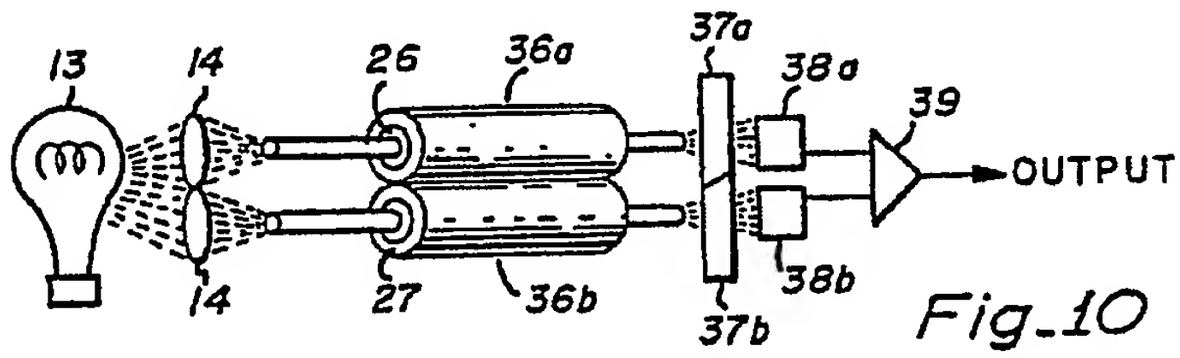

Fig_10

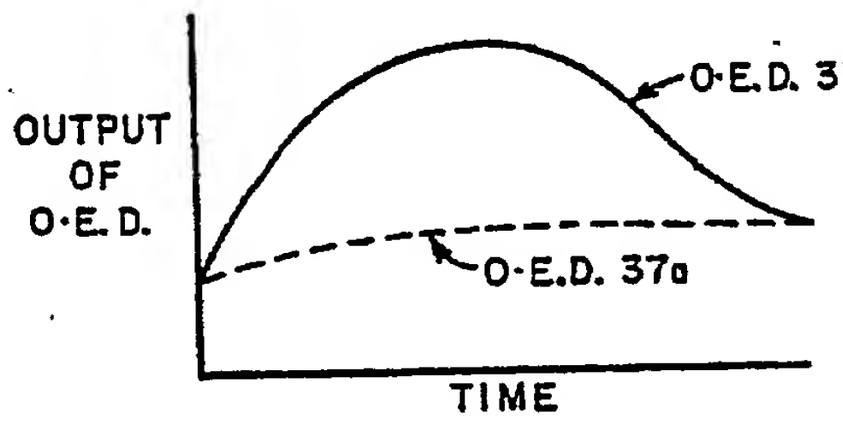

Fig_11

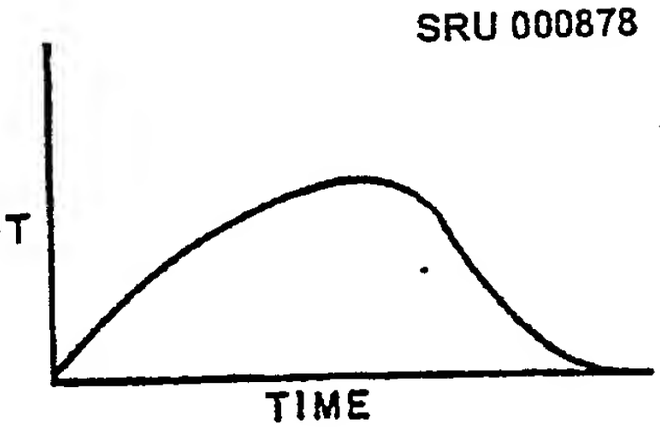

Fig_12

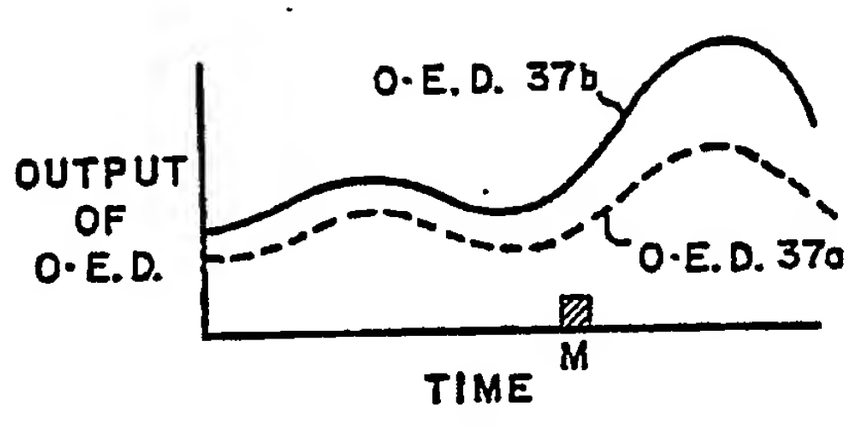

Fig_13

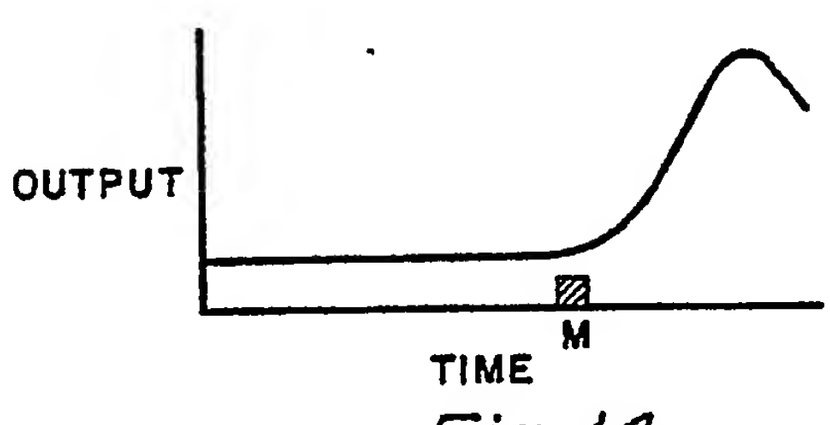

Fig_14

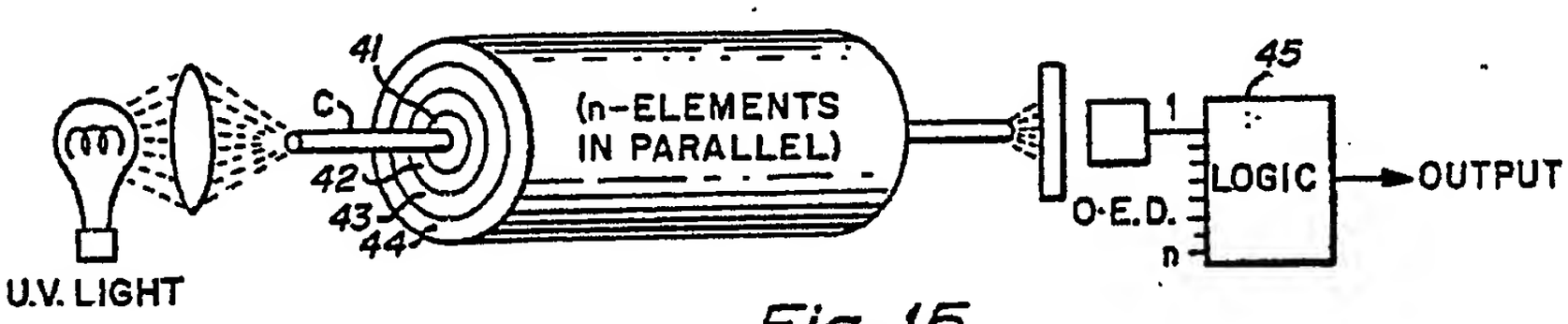

Fig_15

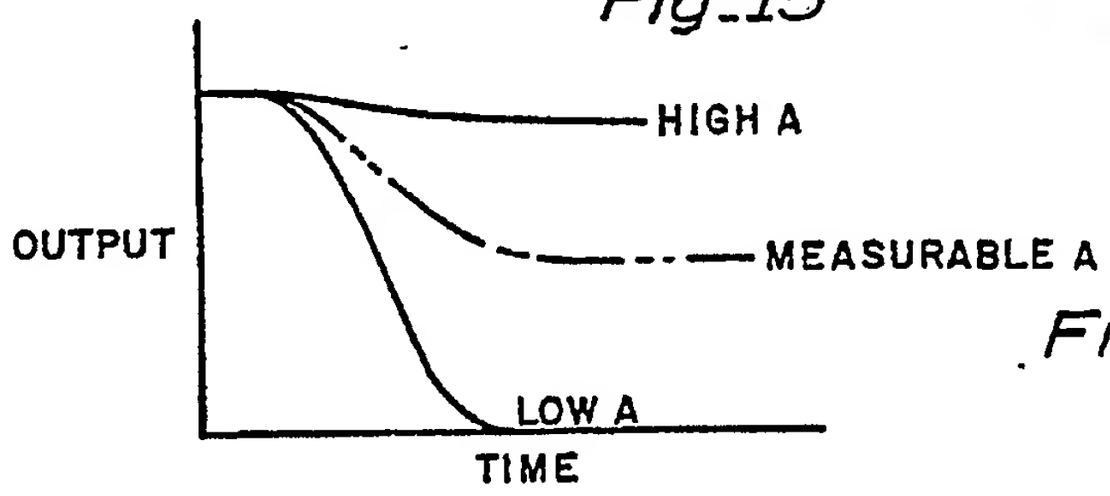

Fig_16

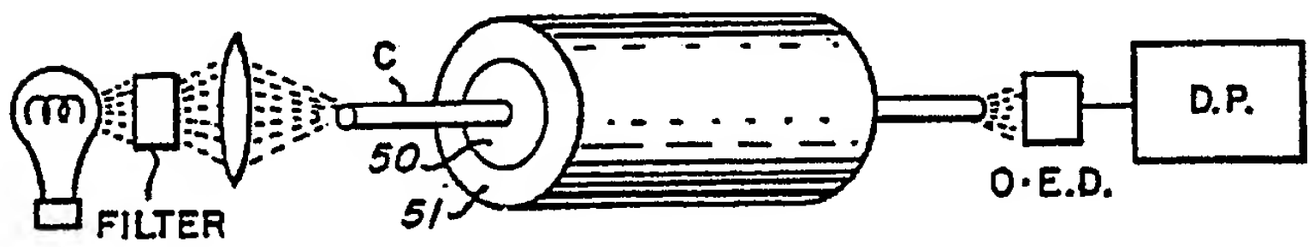

Fig_17

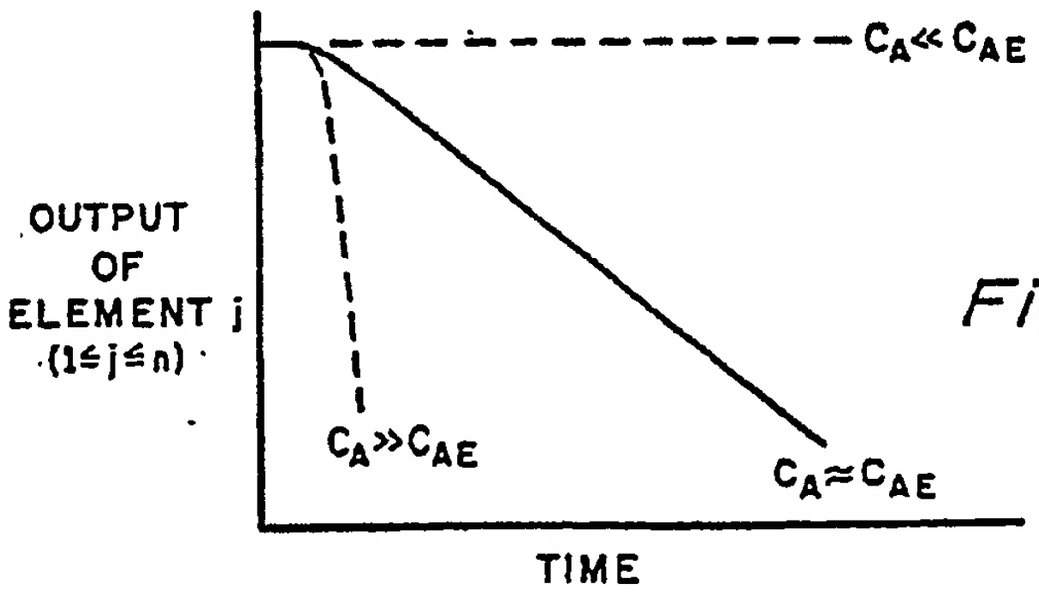

Fig_18

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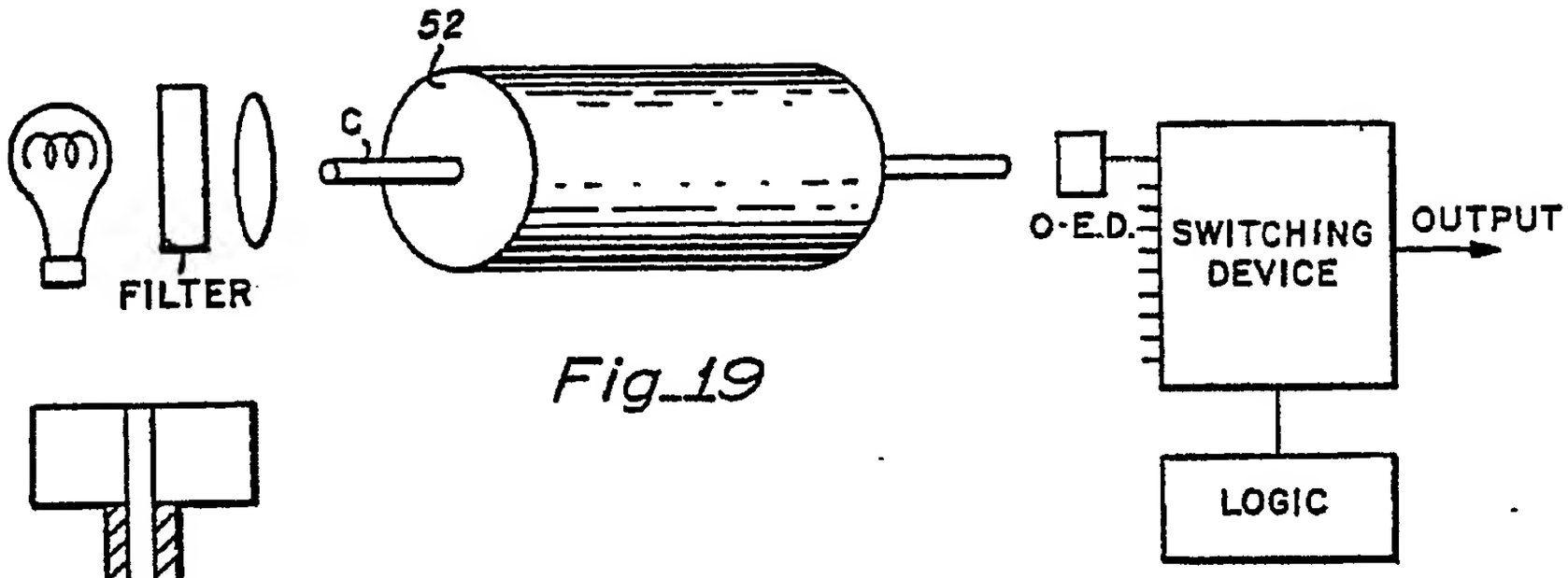

Fig_19

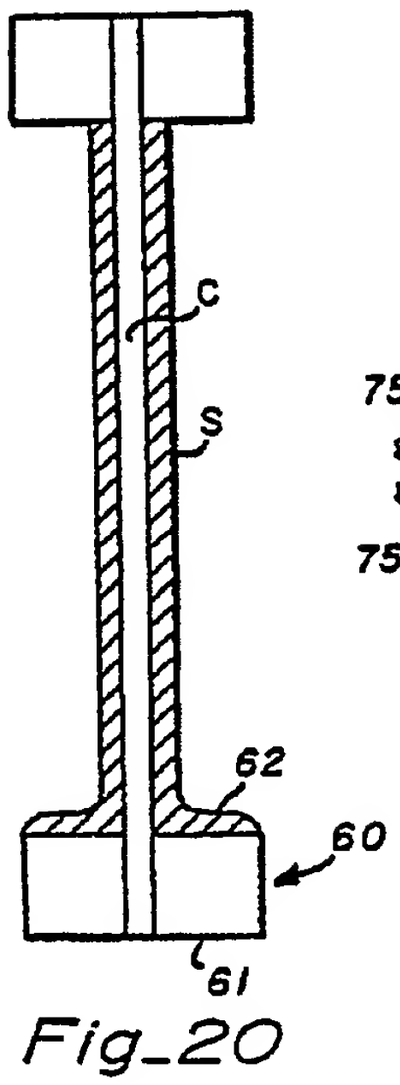

Fig_20

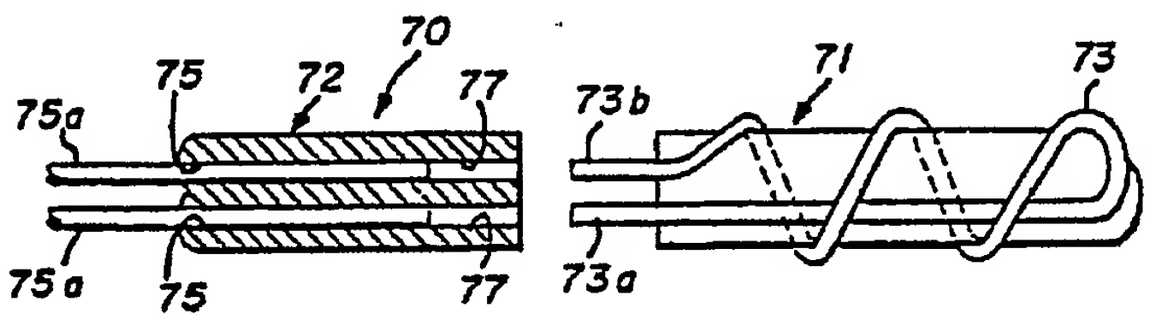

Fig_22

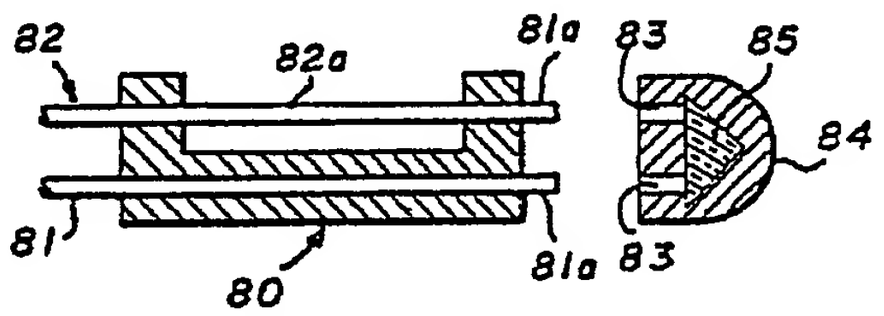

Fig_23

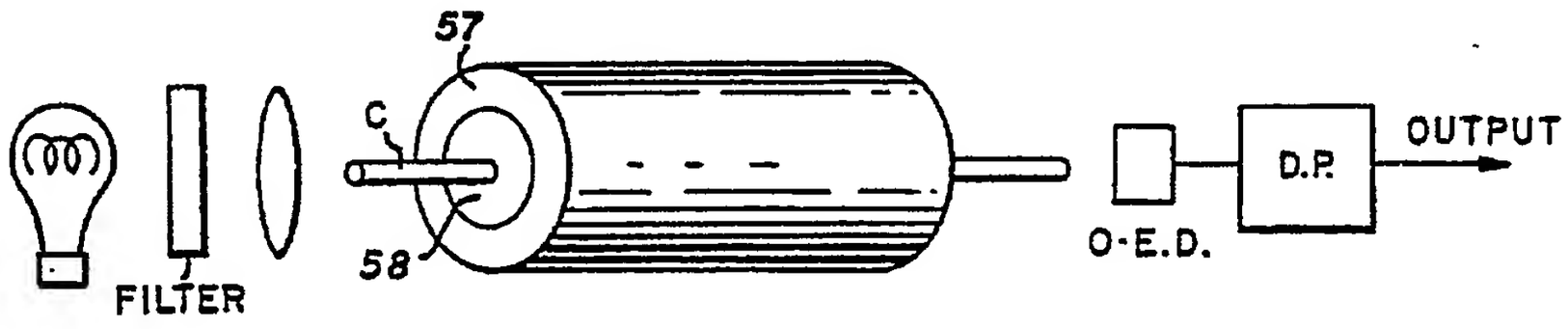

Fig_21

SRU 000880

INTERNATIONAL SEARCH REPORT

International Application No PCT/US80/01210

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL.[3] G01N 31/06,33/48; G01N 21/00 422/55,56,57,58,59,
U.S. CL. 23/230B,230R; 356/435,445,448; 60,68,73

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	23/230B,230R; 356/435,445,448; 422/55,56,57,58,59,60,68,73

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [15]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	US,A, 3,404,962, PUBLISHED 08 OCTOBER 1968, MEDLAR ET AL.	1-35
X	US,A, 4,080,075, PUBLISHED 21 MARCH 1978, BERG.	1-35
A	US,A, 4,099,882, PUBLISHED 11 JULY 1978, ANDRÉN ET AL.	---
X,P	US,A, 4,200,110, PUBLISHED 29 APRIL 1980, PETERSON ET AL.	1-35
X	D E , A , 2744678, PUBLISHED 05 APRIL 1979, SCHAUDEL ET AL.	1-35

[15] Special categories of cited documents: [19]

"A" document defining the general state of the art

"E" earlier document but published on or after the international filing date

"L" document cited for special reason other than those referred to in the other categories

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention

"X" document of *particular relevance*

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
29 DECEMBER 1980	22 JAN 1981
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	BARRY S. RICHMAN